STIC-ILL

TA. P8
Adams

From: Marx, Irene
Sent: Thursday, May 30, 2002 10:14 AM
To: STIC-ILL
Subject: 09/758317

Importance: High

Please send to Irene Marx, Art Unit 1651; CM1, Room 10E05, phone 308-2922, Mail box in 11B01

Faber et al., Pure Appl. Chem. 69, 1613-1632 (1997)

Irene Marx
Art Unit 1651
CMI 10-E-05,
Mail Box 11-B-01
703-308-2922

Pure & Appl. Chem., vol. 69, No. 8, pp. 1613–1631, 1997
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

COMMISSION ON BIOTECHNOLOGY*

BIOTRANSFORMATIONS OF NON-NATURAL COMPOUNDS: STATE OF THE ART AND FUTURE DEVELOPMENT

Prepared for publication by

KURT FABER

Institute of Organic Chemistry, Graz University of Technology, Stremayrgasse 16, A-8010 Graz, Austria

*Membership of the Commission during the work on this document (1996–1997) was as follows:

Chairman: A. Hayes (UK; 1985–97); *Vice-Chairman:* P. M. Norling (USA; 1994–97); *Secretary:* R. Hamelin (France; 1993–96); *Titular Members:* E. Buncel (Canada; 1993–96); F. A. Kuznetsov (Russia; 1995–98); P. Moyna (Uruguay; 1996–99); *Associate Members:* M. C. Chon (Korea; 1995–98); M. J. Droescher (FRG; 1994–97); M. Kawamata (Japan; 1996–99); F. Wang (China; 1994–97); *Ex-officio Member:* J. M. Ward (UK; 1992–97).

Biotransformations of non-natural compounds: State of the art and future development

Abstract: The transformation of non-natural compounds by enzymes—generally referred to as 'biocatalysis'—has evolved as a trend-section of organic synthesis during the mid-eighties. As a consequence, a remarkable number of reliable biochemical techniques have been developed during the last decade, which constitute powerful tools for modern organic synthesis. In this report, the state of the art of biotransformations as well as future developments are critically reviewed with respect to strengths and weaknesses of the existing methods.

INTRODUCTION

The use of Nature's catalysts—enzymes—for the transformation of man-made organic compounds is not at all new as they have been used for about a century (ref. 1). What has changed, is the object of research. Whereas most of the early studies were directed towards the elucidation of biochemical pathways and enzyme mechanisms, the enormous potential for employing enzymes to transform non-natural organic compounds was only realized relatively recently, *i.e.* during the late 1980's.

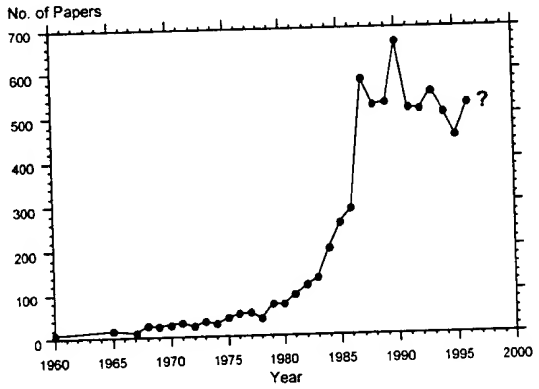


Fig. 1 Annual publications on the biocatalytic transformation of non-natural organic compounds (Note a).

As a result of this intense research, enzymes have captured an important place in contemporary organic synthesis (refs. 2–14). This is reflected by the fact that 8% of all papers on synthetic organic chemistry contained elements of biotransformations already in 1991 (ref. 13) with still an increasing ratio.

Much of the early research was impeded by a tacitly accepted dogma of traditional biochemistry, which stated, that *enzymes are Nature's own catalysts developed during evolution for the regulation of metabolic pathways*. This narrow definition implied, that man-made organic compounds cannot be regarded as substrates. Once this scholastic problem was attacked by non-traditionalists (ref. 15), it was quickly shown that the substrate tolerance of enzymes is much wider than believed. Of course, there are numerous enzymes which are very strictly bound to their natural substrate(s). They play an important role in metabolism and they are generally not applicable for biotransformations (Note b). On the other hand, an impressive number of biocatalysts have been shown to possess a wide substrate tolerance by keeping their exquisite catalytic properties with respect to chemo-, regio- and, most important, enantio-selectivity. For many of these enzymes, the natural substrates—if there are any—are not known. As a consequence, the frequency of use of a particular enzyme is not evenly distributed among the various types of biocatalysts but follows a pattern (Note c) shown in Fig. 2.

Note *a*: Data obtained from database Faber, ~8000 entries (05/1997).
Note *b*: These enzymes are typically employed in biotechnological applications.
Note *c*: Data taken from database Faber, ~8000 entries (05/1997).

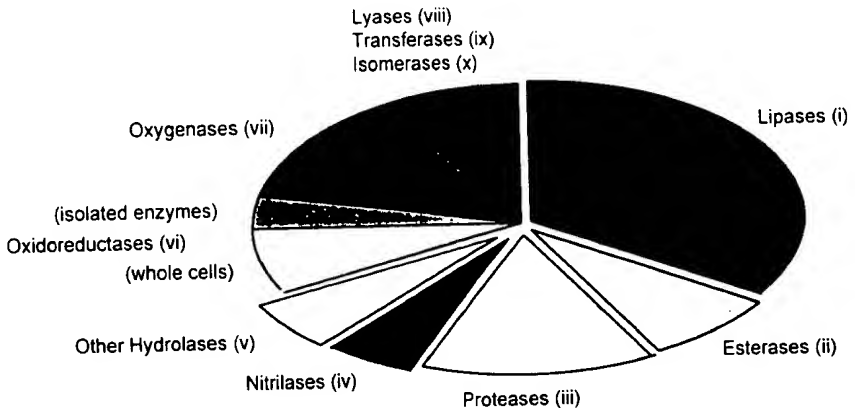


Fig. 2 Frequency of use of enzymes in biotransformations.

(i) Ester formation, -aminolysis, -hydrolysis; (ii) ester hydrolysis; (iii) ester and amide hydrolysis, peptide synthesis; (iv) nitrile hydrolysis; (v) hydrolysis of epoxides, halogens, phosphates, glycosylation; (vi) reduction of aldehydes, ketones and enoates; (vii) biohydroxylation, sulfoxidation, epoxidation, Baeyer-Villiger oxidation, dihydroxylation; (viii) cyanohydrin formation, acyloin and aldol reaction; (ix) glycosyl transfer; (x) Claisen-type rearrangement, isomerization of carbohydrates, racemization and epimerization.

In this report, a brief summary is given on the state of the art of biotransformations (refs. 16, 17) with special emphasis on the general applicability and reliability of various reaction types, by following the reaction principles shown in Fig. 2.

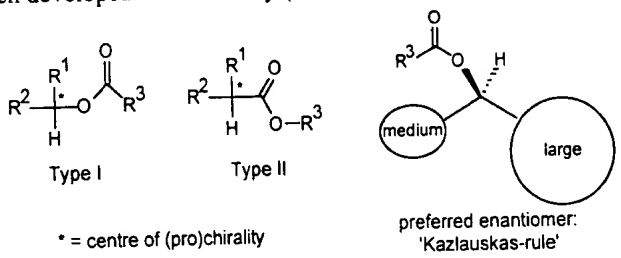
HYDROLASES

Reactions catalyzed by various types of hydrolases are predominant among biotransformations. The lack of sensitive cofactors, which have to be recycled, makes them particularly attractive for organic synthesis. Consequently, they account for about two thirds of all reactions reported. In particular, reactions involving the cleavage (or formation) of an amide- or ester bond are most easy to perform by using lipases, esterases and proteases, respectively. Other types of hydrolysis reactions involving phosphate esters, epoxides, organo-halogens and nitriles are still hampered by a restricted availability of enzymes, but they hold great synthetic potential.

Lipases

A large number of fat-cleaving enzymes—lipases—are produced on an industrial scale for applications in the food and detergent industry (ref. 18). This is facilitated by the fact that many of them are formed extracellularly by fungi and bacteria. This ready availability has created an enormous spin-off with respect to the enantioselective hydrolysis and formation of carboxyl esters (ref. 19). Bearing in mind that the natural substrates for lipases—glycerides—possess a chiral alcohol moiety, it is understandable, that lipases are particularly useful for the resolution or asymmetrization of esters bearing a chiral alcohol moiety (Fig. 3). From the data available, the following general guidelines for the design of lipase-substrates can be given: (i) The center of chirality should be located as close as possible to the site of the reaction (*i.e.* the ester carbonyl group) to ensure an optimal chiral recognition. Thus, esters of secondary alcohols are usually more selectively transformed than those of primary alcohols. (ii) There is a wide tolerance for the nature of both substituents R^1 and R^2, but they should differ in size and/or polarity to aid the chiral recognition process. They may be also linked together to form cyclic structures. Polar groups, such as carboxylate, amide or amine—which would be heavily hydrated in an aqueous environment—are not tolerated and, if they are required, they should be protected with a lipophilic unit. (iii) The alkyl chain of the acid moiety (R^3) should be preferably of straight-chain nature possessing at least three to four carbon atoms. Insufficient reaction rates may be improved by using 'activated' esters bearing haloalkyl groups, *e.g.* Cl-CH_2- and Cl-$(CH_2)_2$- for Type I and II, respectively. (iv) The remaining hydrogen atom in both substrate types must not be replaced by a substituent, since esters of tertiary alcohols and α,α,α-

trisubstituted carboxylates are usually not accepted by lipases. (v) The stereochemical preference of the most commonly used lipases (*e.g.* from *Pseudomonas* and *Candida* spp.) for esters of secondary alcohols follows an empirical model generally referred to as 'Kazlauskas-rule' (ref. 20). A related rationale for primary alcohols has been developed more recently (ref. 21).



Enzyme type	Preferred substrate type	R^1, R^2	R^3
Lipases	Type I > Type II	wide tolerance of (functionalized) alkyl and aryl; different in size, not too polar.	long-chain ($\geq n$-C_3H_7)
Esterases and proteases	Type II > Type I		short-chain ($CH_3 > C_2H_5$)

Fig. 3 Substrate types for lipases, esterases or proteases.

The strength of lipases is the ample availability of bulk-enzymes from various microbial sources. It can be anticipated that almost every secondary alcohol can be resolved by employing lipase-technology by following the general guidelines described above. Whereas the stereochemical preference for one enantiomer of a given substrate (a 'digital' decision) can be predicted with fair accuracy, the stereoselectivity (Note d) (*i.e.* an 'analog' value) has to be determined in practice. Despite an impressive effort in molecular modelling, attempts to develop reliable methods for the prediction of lipase-stereoselectivity have failed so far. One of the reasons for this obvious weakness of lipase-technology is the fact that these studies have (almost invariably) been based on the X-ray structure of lipases which provides only *static* information. Bearing in mind that the enzyme–substrate interaction consists of a considerable *dynamic* nature, it can be assumed that the application of other techniques, such as NMR- and fluorescence-spectroscopy may help to provide solutions.

The high stability of lipases towards organic solvents makes them extremely useful for the reverse reaction, *i.e.* ester formation by condensation or (more advantageous) transesterification reactions (refs. 22, 23). The use of non-natural nucleophiles in acyl-transfer reactions such as amines, ammonia, hydrazine, oximes, and hydrogen peroxide allows the lipase-catalyzed aminolysis, ammonolysis (ref. 24), hydrazinolysis (ref. 25), oximolysis and perhydrolysis (ref. 26) of esters giving rise to carboxamides, hydrazides, hydroxamic acids and peracids, respectively. Whereas hydrazine and oxime derivatives have been used more scarcely, lipase-catalyzed aminolysis and perhydrolysis is particularly advantageous for those cases where traditional chemical catalysis fails and where side reactions dominate.

The use of organic media at low water activity offers a unique possibility to tune stereoselectivity through variation of the solvent (Note e). Bearing in mind that enzymes possess delicate and soft structures, it may be anticipated that any solvent exerts a significant influence on the catalytic properties of an enzyme, such as reaction rate, and its various types of selectivities. Thus, it can be expected that an enzyme's specificity can be controlled by varying the solvent's properties (ref. 28). For reactions performed in water, however, this is hardly possible, because its physicochemical properties are determined by Nature and can be altered only within a very narrow margin. On the other hand, an organic solvent can be chosen within certain limits (Note f) from a wide arsenal having different properties such as dipole moment, water-solubility, straight-chain or cyclic structure, flexibility and the ability to form

Note *d*: Usually expressed as the ratio of the relative rate constants of the enantiomers, the 'Enantiomeric Ratio' (*E*) (ref. 27).
Note *e*: This technique is generally referred to as 'medium-engineering'.
Note *f*: From a number of methods to estimate the compatibility of an organic solvent ensuring sufficient enzyme activity, the log *P*-value has been shown to be most reliable. As a rule of thumb, an organic solvent having a log *P* of ≥ 1 should not deactivate a reasonably stable enzyme.

hydrogen bonds (ref. 29). As a consequence, the stereoselectivity of an enzyme-catalyzed reaction—this is particularly true for lipases—can be controlled by choosing the appropriate organic solvent. Any attempts to predict these effects, however, have failed so far and the selectivity-enhancement of lipase catalyzed reactions by medium engineering is still a largely empirical task, despite the tremendous amount of data published to date. However, the empirically obtained selectivity-improvement rates are often impressive and even a complete reversal of stereochemical preference is possible (ref. 30).

At present, lipases are continuously used for the generation of enantiomerically enriched primary and secondary alcohols and—to a somewhat lesser extent—of chiral carboxylic acids and secondary amines. Several useful techniques for the selectivitity-enhancement are available to turn lipase-technology into a reliable tool for the creation of chiral synthons (ref. 31). However, limitations with respect to the predictability of stereoselectivity will persist for some time.

Esterases

In contrast to the large number of commercially available microbial lipases, less than a handful of true esterases are available (ref. 32). The large majority of esterase-catalyzed reactions have been performed by using porcine liver esterase (PLE) (ref. 33). This enzyme has been widely used for the hydrolysis of Type II esters (Fig. 2) with R^3 being preferably methyl or ethyl, whilst Type I substrates (employed as the acetate esters) have been used to a lesser extent. In comparison to lipases, the applicability of PLE is significantly restricted to reactions performed in an aqueous medium, as PLE has been shown to exhibit low activity and erratic results with respect to stereoselectivity when placed in (nearly) anhydrous organic solvents (Note g). Thus, in contrast to lipases, selectivity-tuning is limited to the addition of water-miscible organic co-solvents, which only can be used in fractions of about up to 20% of volume (ref. 31). Other esterases, such as the enzyme from horse or rabbit liver have been shown to possess a related and often slightly altered substrate specificity as compared to PLE (ref. 34). In general terms, they are of limited use. Likewise, the applications of acetylcholine esterase are also few in number due to the fact that this enzyme is predominantly isolated from the electric eel which makes it prohibitively expensive, particularly for the production scale. The enantioselectivities achieved, however, are often excellent (ref. 36). Cholesterol esterase is also of limited use, since it seems to work only on relatively bulky substrates, which show some similarities to its natural substrates, *i.e.* steroid esters (ref. 37).

To overcome the narrow range of readily available esterases (Note h), whole microbial cells have been used instead of isolated enzyme preparations (ref. 38). Although some surprising highly selective transformations have been reported, this technique is of limited use because the nature of the active enzyme system is unknown and, as a consequence, optimization is a complicated task. One of the few available microbial esterases is carboxyl esterase NP (Note i). This enzyme was obtained from a *Bacillus subtilis* sp., which was identified through extensive screening aiming at a highly selective catalyst for the resolution of α-aryl propionic acids, in particular aiming at the anti-inflammatory agent naproxen (ref. 39). Carboxyl esterase NP accepts a variety of substrates, but it exhibits optimal activity and selectivity when the carboxyl ester substrate has an aromatic side chain (ref. 40).

Fortunately, a large number of proteases can be used to selectively hydrolyze carboxylic acid esters (Note j) and this effectively compensates for the limited number of readily available esterases (ref. 4). The most frequently used members of this group are α-chymotrypsin, subtilisin and—to a lesser extent—trypsin, pepsin, papain and penicillin acylase (ref. 41). As a rule of thumb, with carboxyl esters, most proteases seem to retain a preference for the hydrolysis of that enantiomer which mimics the configuration of an L-amino acid more closely. It is a common observation, that proteases and esterases often exhibit an

Note *g*: This problem has been circumvented recently by using chemically modified PLE (ref. 35).
Note *h*: Only recently, esterases from various sources such as *Bacillus* sp., *Mucor miehei* and from *Thermoanaerobium brockii* have become available from commercial sources. However, since there are few data on their substrate-selectivity pattern hitherto available, they have to be used as 'black-box'-enzymes at present.
Note *i*: NP stands for the anti-inflammatory agent 'naproxen'. The enzyme has been made available through genetic engineering.
Note *j*: Bearing in mind the greater stability of amides as compared to esters, it is understandable, that many proteases can also hydrolyze esters. On the other hand, carboxamides cannot be cleaved by carboxyl ester hydrolases such as lipases and esterases. However, there are few exceptions to this rule, such as the PLE-catalyzed hydrolysis of highly strained β-lactams.

opposite stereochemical preference when acting on α-chiral carboxylic esters. The latter phenomenon may be explained by the fact that the active site serine residues of acetylcholine esterase and several serine proteases are located at opposite sides of the active site (ref. 42).

The general rules for the substrate-construction for esterases and proteases are closely related to those for lipases (Fig. 2). However, R^3 should preferably be a short-chain unit, leading to acetates (Type I) and methyl carboxylates (Type II), respectively. The fact that the chiral recognition of esterases and proteases for α-chiral carboxylates is often high makes them complementary to lipases to a certain extent. The latter fact counts for their strength. However, the number of commercially available esterases is small and their applicability in organic solvents is limited. Thus, the development of novel esterases from microbial sources is a worthwhile endeavour.

Proteases

The enzymatic hydrolysis of the carboxamide bond is naturally linked to amino acid and peptide chemistry. Although the top amino acids on the world market (with respect to annual production and value) are produced mainly by fermentation (Note k), an increasing number of optically pure L-amino acids are produced on an industrial scale by an enzymatic method to be used as additives for infusion solutions and as enantiopure starting materials for the synthesis of pharmaceuticals, agrochemicals and artificial sweeteners, such as Aspartame. On the other hand, a small (but important) number of D-configured amino acids are used as bioactive compounds or components of such agents. For instance, D-phenylglycine and its *p*-hydroxy derivative are used for the synthesis of antibiotics such as ampicillin and amoxycillin, respectively, and D-valine is an essential component of the synthetic pyrethroid fluvalinate (ref. 43).

The best two methods of general applicability are shown in Fig. 4. (i) The amidase-method makes use of L-selective amidases from *Pseudomonas*, *Aspergillus* or *Rhodococcus* sp., which hydrolyze L-amino acid amides from a racemate with excellent specificity (ref. 44). The possibility to recycle the unreacted D-configured amide *via* its corresponding Schiff-base with benzaldehyde in a separate step makes this procedure economical.

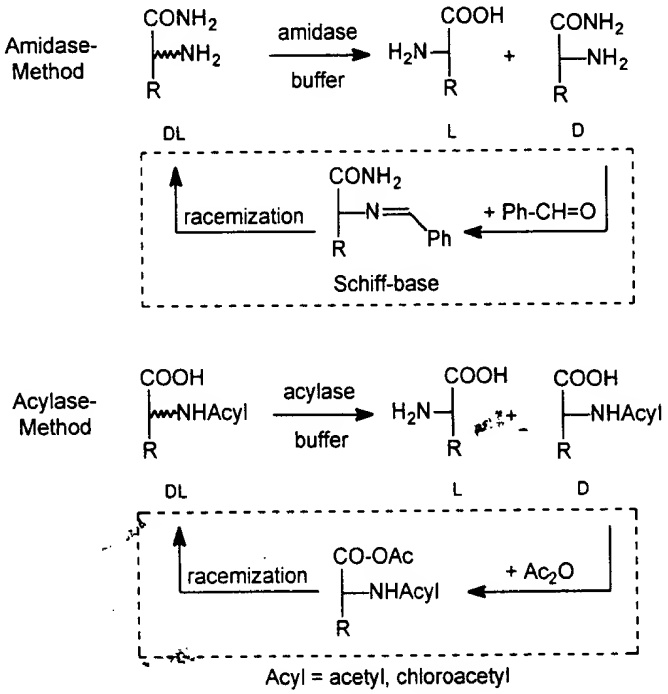
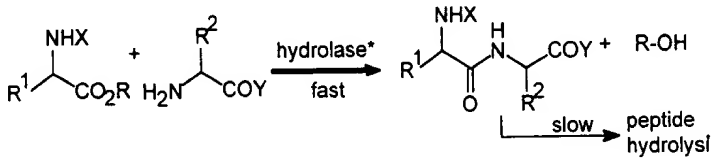


Fig. 4 Resolution of amino acids *via* the amidase- and acylase-method .

Note *k*: L-Glutamic acid, L-lysine and D,L-methionine.

(ii) The acylase method uses *rac*-*N*-acyl amino acids as substrates (ref. 45). L-amino acids are liberated by the action of an acylase from porcine kidney (Note l) or from *Aspergillus* or *Penicillium* sp. The versatility of this method has been demonstrated by the resolution of *N*-acetyl tryptophan and -phenylalanine on an industrial scale using immobilized enzymes in column reactors. Again, in order to ensure a commercial success, the non-reacting D-enantiomer may be recycled *via* racemization of the corresponding mixed anhydride intermediate in a separate step.

Peptides have gained increasing attention due to their diverse biological activity. They may be used as sweeteners, antibiotics and chemotactic agents, as well as growth factors and they play an important role in hormone release by acting either as stimulators or inhibitors. More recently, they are used as immunogens for the creation of specific antisera. The most frequently used methods of peptide synthesis are purely chemical in nature which is impeded by two drawbacks, *i.e.* (i) potential of racemization, particularly during the activation step and (ii) tedious purification of the final product from isomeric peptides with a closely related sequence. To circumvent these problems, peptide synthesis is increasingly carried out enzymatically by using proteases (Note m) or lipases and esterases (Note n) (ref. 46). From the various basic ways to achive peptide synthesis—reversal of hydrolysis, transpeptidation and aminolysis of esters (Note o)—the latter method is used most frequently (ref. 47).



X = N-terminal blocking group (e.g. Ph-CH_2-O-CO-, *t*-Bu-O-CO-)
Y, Z = C-terminal blocking group (e.g. *t*-Bu-, Ph-CH_2-, Ph-NH-NH-)
R = leaving group (e.g. Me, Et, haloethyl)
* esterase, lipase or protease

Fig. 5 Enzymatic peptide synthesis using ester aminolysis.

Since the peptide formed during ester aminolysis can be hydrolytically cleaved in a subsequent side reaction in the presence of water, these reactions have to be terminated before equilibrium is reached. In addition, undesired peptide hydrolysis may be efficiently suppressed by performing the reaction in frozen aqueous medium at -15° C (ref. 48) or by using non-proteolytic enzymes, such as esterases or lipases (ref. 49). The key to enzymatic peptide synthesis is the availability of enzymes which can potentially cover all possible types of peptide bonds. Although the existing range of proteases is far from complete, reasonably coverage is provided. The most striking shortage involves proline derivatives.

The exquisite specificity of proteases for L-configured substrates turns into a drawback when the incorporation of D-amino acids is required. Although attempts have been reported to 'weaken' the L-specificity of proteases by solvent engineering or by enzyme-modification on the genetic level, no real solution has been provided to solve this limitation.

Nitrilases and nitrile hydratases

Bearing in mind that many organic nitriles are synthesized by plants, fungi, bacteria, algae, insects and sponges, it is not surprising, that there are several biochemical pathways for nitrile degradation. From a preparative standpoint, the most interesting among them involves nitrile hydrolysis (Fig. 6) (ref. 51). The latter may take place *via* two different pathways: (i) Single-step hydrolysis is catalyzed by a nitrilase yielding the corresponding carboxylic acid, whereas (ii) a two-step process involving the intermediate

Note *l*: Recommended for laboratory scale reactions (ref. 50).
Note *m*: Occasionally, proteases used for peptide synthesis are also misleadingly called 'peptide ligases'; they are, however, simple hydrolases and have nothing in common with class 6-enzymes.
Note *n*: Since in principle every hydrolase, which acts *via* an acyl-enzyme intermediate can catalyze the aminolysis of esters, lipases and esterases can be used for the enzymatic peptide synthesis *via* ester aminolysis. On the other hand, metallo- or carboxyproteases are inapplicable for this method.
Note *o*: Reversal of hydrolysis and transpeptidation is impeded by limited yields due to thermodynamic control. Kinetic control in ester aminolysis gives considerably higher yields.

formation of the carboxamide is catalyzed by a nitrile hydratase and an amidase (Note p), respectively. Both types of nitrile-converting enzymes act *via* distinctively different mechanisms. Until recently, it was assumed, that the pathway for nitrile hydrolysis is mainly dependent on the substrate structure and that the outcome of a given reaction can be predetermined to a certain extent—*i.e.* aromatic, unsaturated and heteroaromatic nitriles are hydrolyzed *via* nitrilases, whereas aliphatic counterparts are transformed through two steps. In view of recent findings, however, this distinction should be regarded as less well defined and has to be investigated on a case-to-case basis (ref. 52).

From a synthetic viewpoint, cyanide represents a widely applicable C_1-synthon (Note q), which can be employed for the homologation of a carbon framework, on the other hand, further transformations of the nitrile thus obtained are impeded due to the harsh reaction conditions required for its hydrolysis. In this context, the chemo-selective biocatalytic hydrolysis of nitriles represents a valuable alternative because it occurs at ambient temperature and near physiological pH. One of the most impressive story of success is the production of acrylamide by hydration of acrylonitrile using whole bacterial cells. This biotransformation is performed on an industrial scale with annual amounts exceeding 30,000 tonnes. Yields of >99% are achieved and the formation of by-products such as acrylic acid, which plagues traditional methodology, is completely avoided (ref. 53). Beginning 1998, the same biocatalyst will also be used in China for the annual production of 3,000 tonnes of nicotinamide from 3-cyanopyridine (ref. 54).

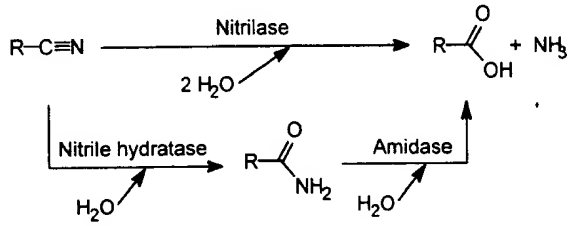


Fig. 6 Pathways for enzymatic nitrile hydrolysis.

Regio-selective hydrolysis of one nitrile group out of several in a molecule is generally impossible using traditional catalysts which lead to the formation of complex mixtures, but whole microbial cells can be very effectively used for this purpose (ref. 55). As a rule of thumb, the mono-hydrolysis of a dinitrile yields a more polar cyano-carboxylic acid or -amide, which is not a substrate for nitrile-converting enzymes. The strength of enzymatic nitrile-hydrolysis lies predominantly in chemo- or regio-selective transformations. With respect to enantio-selectivity, nitrilases and nitrile-hydratases are generally poor catalysts. Although a limited number of enantio-selective hydrolyses have been reported, in the majority of cases the enantio-selection was shown to be linked to the amidase (ref. 56). Furthermore, there is no nitrile-hydrolysing enzyme preparation commercially available (Note r) at present and reactions have to be performed by using whole cells (Note s).

Epoxide hydrolases

The asymmetric hydrolysis of an epoxide provides provides access to chiral 1,2-diols and (in kinetic resolutions) also to enantiomerically enriched epoxides. Both of these materials are widely used as highly valuable intermediates (Note t) for asymmetric synthesis (ref. 57). At present, asymmetric epoxide hydrolysis can only be achieved by biocatalytic methods. Whereas epoxide hydrolases from mammalian sources such as rat or rabbit liver have been extensively investigated aiming at the elucidation of detoxification studies (ref. 58), they are of limited use due to poor availability (Note u). On the other hand, enzymes from microbial sources such as bacteria and fungi have been identified only recently (ref. 59). The

Note *p*: Amidases are a subgroup of proteases.
Note *q*: Cyanide is one of the few water-stable carbanions.
Note *r*: A crude immobilized enzyme preparation derived from a *Rhodococcus* sp. was available from Novo Co. (DK) for some time (depending on the carrier material denoted as SP 409 and SP 361), but it has been withdrawn recently.
Note *s*: As a strain of general applicability, *Brevibacterium* R312 (recently reclassified as *Rhodococcus* R312) can be recommended.
Note *t*: Vicinal diols are employed as the corresponding cyclic sulfite or sulfate esters.
Note *u*: Reactions catalyzed by hepatic epoxide hydrolases rarely surpass the millimolar scale (ref. 64).

latter can be produced on an (almost) unlimited scale by simple fermentation (Note v). In principle, the asymmetric biocatalytic hydrolysis of an epoxide may be conducted in various ways (Fig. 7): (i) Kinetic resolution provides the corresponding 1,2-diol and the residual substrate in 50% theoretical yield. (ii) Alternatively, enantioconvergent hydrolysis leads to the vicinal diol as the sole product in 100% theoretical yield. The latter transformation may be achieved by using (iia) two different biocatalysts possessing opposite enantio- and regio-selectivity (ref. 60) or (iib) by using a bio- and chemo-catalytic step in a sequence (ref. 61). The strength of enzymatic epoxide hydrolysis has recently tremendously increased due to the availability of microbial strains showing sufficient activity (refs. 62, 63). Although these reactions show a strongly impirical aspect—*i.e.* knowledge on the substrate-selectivity pattern is limited at present—this area is heavily investigated and it can be anticipated that methods of great potential will be provided.

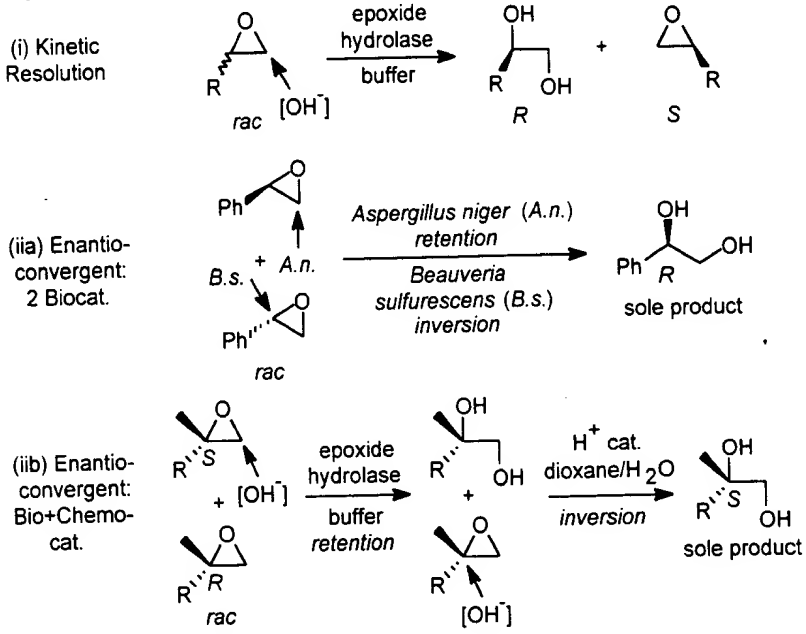


Fig. 7 Biocatalytic hydrolysis of epoxides *via* kinetic resolution or through enantio-convergent processes.

Phosphatases and kinases

The hydrolysis of phosphate esters can be achieved by chemical methods and the application of enzymes—phosphatases—is only advantageous if the substrate is susceptible to decomposition (ref. 65). Thus, the enzymatic hydrolysis of phosphate-esters has found only a limited number of applications, bearing in mind that the asymmetric transformation of an alcohol *via* an ester-like derivative is much more convenient by employing a lipase, esterase or protease (ref. 19). On the contrary, phosphate ester *formation* is of considerable importance—in particular when regio- or enantioselectivity is required—because numerous bioactive agents display their highest activity only when they are transformed into phosphorylated analogues. Thus the phosphorylation of OH-groups at the expense of adenosine triphosphate (ATP) by using kinases has been developed to a great extent (ref. 66). A number of kinases are available and from various methods for ATP-recycling, the system based on pyruvate kinase/phosphoenol pyruvate (PEP) is probably the most useful (ref. 67). PEP is not only very stable towards hydrolysis but it is also a stronger phosphorylating agent than ATP, thus driving the phosphorylation reaction to completion. Additionally, nucleosides other than adenosine phosphates are also accepted by pyruvate kinase.

Dehalogenases and halohydrin epoxidases

The replacement of halogen with hydroxyl in a formal hydrolysis reaction is catalyzed by dehalogenases (Note w) (ref. 68). Depending on the enzyme used, the reaction usually proceeds with inversion of

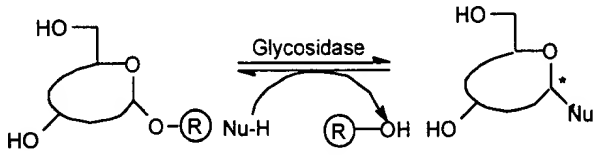
Note *v*: Microbial epoxide hydrolases are currently developed by Fluka Chemie (CH).
Note *w*: Dehalogenases are classified depending on their substrate type; for biotransformations, haloalkane dehalogenase and α-haloacid dehalogenase are most important.

configuration, *e.g.* L-α-chloropropionic acid yields D-lactate, and *vice versa* (ref. 69). Although these enzymes would hold great synthetic potential, they are not widely distributed in Nature and most of the studies from this area are concerned with the degradation of xenobiotics rather than synthetic biotransformations.

On the other hand, the formal elimination of hydrogen halide from a halohydrin under catalysis of a halohydrin epoxidase yields an epoxide. The bulk of research published so far has shown that these enzymes exhibit low enantio-selectivity in general (ref. 70).

Glycosidases

In Nature, glycosidases (Note x) are responsible for the hydrolytic breakdown of oligo- and poly-saccharides. Since the latter reaction is of no use to the synthetic chemist, the reverse reaction—glycosylation—had been investigated with considerable intensity. Two principles, which are related to the formation of peptides from amino acid (derivatives), have been developed (ref. 71): (i) glycosylation by reverse hydrolysis and (ii) transglycosylation (ref. 72). Enzymatic glycosylation is plagued by a central dilemma—equilibrium. *Pushing* the reaction by using one of the carbohydrate-components in large excess leads to syrup-like reaction mixtures which are difficult to handle and reactions are impossible to control.



Nu = Nucleophile: primary or secondary alcohol, sugar
* Anomeric Center

Reverse Hydrolysis	Transglycosylation
R = H	RO = F-, glycosyl-, Ph-, *p*-NO_2-C_6H_4-.

Fig. 8 Principles of enzymatic glycosylation *via* reverse hydrolysis and transglycosylation.

On the other hand, one can *pull* the equilibrium by removing the water (or the leaving group R-OH) formed. This approach would work perfectly well if lipophilic organic solvents such as toluene or ethers could be employed (cf. the ester synthesis using lipases). Unfortunately, carbohydrates are only sparingly soluble in such solvents and more polar substitutes such as acetone, dimethylformamide (DMF) and dimethylsulfoxide (DMSO) have to be used (ref. 73). The latter, however, lead to enzyme deactivation and only the most stable glycosidases can be used. As a consequence, despite all these tricks, yields are often less than perfect. The strength of enzymatic glycosylation using glycosidases is the perfect stereo-control of the newly formed anomeric centre (Note y) by chosing one of the many available enzymes with matching selectivity, *i.e.* an α- or β-glycosidase. On the other hand, regio-control with respect to the hydroxyl group being glycosylated (on the acceptor) is often low which results in regio-isomeric product mixtures which are difficult to purify. Parallel to low regio-selectivity, diastereo-control is also generally low in cases where a racemic, prochiral- or *meso*-acceptor is employed (ref. 74). At present, it cannot be forseen whether these drawbacks will be surmounted in the near future.

REDOX REACTIONS

Dehydrogenases

The asymmetric reduction of ketones or aldehydes possessing chiral centre(s), can be performed by using isolated dehydrogenases (ref. 75) or whole microbial cells (Note z) (ref. 76) yielding the corresponding secondary of primary alcohols, respectively. For ketones, the stereochemical outcome of the reaction can be predicted with good accuracy—it is mainly determined by the nature of the enzyme and by the steric requirements of the substrate. This rationale is generally referred to as 'Prelog's rule' (ref. 77). To provide

Note *x*: Glycosidases are also termed 'glycohydrolases'.
Note *y*: This phenomenon is commonly denoted as 'anomeric control'.
Note *z*: Fungi are more popular than bacteria.

access to both enantiomeric products, the availability of dehydrogenase enzymes showing opposite stereoselectivites—*i.e.* Prelog and anti-Prelog specificity—is required. At present, the majority of dehydrogenases lies definetely in favor of Prelog-enzymes (Note aa) , while complementary candidates (Note ab) are more difficult to obtain. Another weakness of dehydrogenases originates from the requirement of a redox-cofactor—*i.e.* depending on the enzyme, either reduced nicotinamide adenine dinucleotide (NADH) or its phosphorylated analogue (NADPH). Whereas an excellent method for the recycling of NADH has been developed by using formate dehydrogenase (from *Candida boidinii*) and formate as the ultimate reductant (ref. 78), NADPH-recycling is more complicated. Only recently, a formate dehydrogenase of wider applicability from *Pseudomonas* sp. has been developed through site-directed mutagenesis (ref. 79). In contrast to the *Candida* enzyme, the latter is able to accept both NADH and NADPH as substrate.

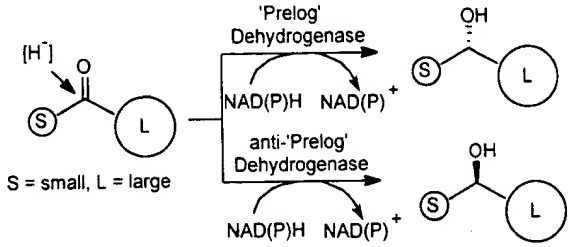


Fig. 9 Asymmetric reduction of ketones with complementary selectivities.

However, cofactor-recycling itself can be avoided by using whole-cell systems (ref. 760). On the one hand, there are sufficient strains known to possess the desired opposite stereo-specificities, but, in contrast, isolated yields are usually less than optimal and reproducibility is limited. Furthermore, optical purities of product may vary due to the many (often unknown) enzymes which may be involved in the transformation.

Depending on the substitution pattern of the substrate, the asymmetric reduction of activated carbon carbon double bonds (Note ac) may lead to the formation of one or two new stereocenters. This highly valuable synthetic reaction can be performed by using NADH-dependent enoate reductases, which are found in many microorganisms such as *Clostridia* and methanogenic *Proteus* sp. (ref. 80) and even in baker's yeast (ref. 76). Although several enzymes of this type have been isolated and characterized, the majority of preparative-scale biotransformations have been performed by using whole-cell systems. The latter is mainly due to the sensitivity of enoate reductases to traces of oxygen. The strength of this method lies in its simplicity—as far as the handling of baker's yeast is regarded—but it is not without drawbacks which are typical for whole-cell systems, such as undesired side-reactions (*e.g.* over-reduction) due to the presence of a multitide of enzymes being involved and low overall yields. It is unlikely, that enoate reductases will be available for use in an isolated form.

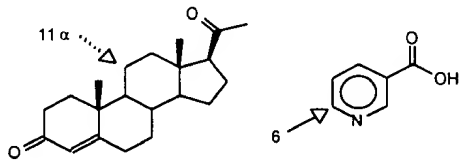
Oxygenation

There is one severe limitation to (almost) all chemical oxidation processes: the cheapest oxidant — molecular oxygen—cannot efficiently be employed for the group-selective oxidation of organic molecules. Chemical substitutes are often based on toxic metal ions, such as Cr, or unstable peroxo-compounds such as hydroperoxides and peroxycarboxylic acids. As a consequence, halogenated intermediates have been widely used as temporary substitutes for oxygenated species, which has led to severe problems in waste-treatment due to recalcitrant organohalogen-compounds. Since oxygenation is a common reaction in Nature, biotransformations can offer some valuable alternatives (ref. 81).

Enzymes which introduce one oxygen atom from molecular oxygen into a substrate—mono-oxygenases—can catalyze a variety of synthetically useful reactions, such as (i) hydroxylation of aliphatics, (ii) epoxidation of alkenes, (iii) sulfoxidation of thioethers and (iv) Baeyer-Villiger oxidation of ketones. All of these reactions can potentially be performed in an asymmetric fashion. One severe limitation which is common to (almost) all mono-oxygenase reactions is their requirement of a cofactor,

Note *aa*: For instance, alcohol dehydrogenases from yeast, horse liver, *Thermoanaerobium brockii* or *Curvularia falcata* and hydroxysteroid dehydrogenase.
Note *ab*: For instance, alcohol dehydrogenases from *Lactobacillus kefir*, *Mucor javanicus* or *Pseudomonas* sp.
Note *ac*: For instance, in α,β-position to an alcohol, ketone, carboxylic acid or ester, lactone, nitro-groups, etc.

such as NADH or (more commonly) NADPH. Since the recycling of the latter is particularly cumbersome (see above), the majority of mono-oxygenase reactions are performed by using whole-cell systems. Since the oxidation step often constitutes the first difficult step—the bottleneck—for the complete metabolic degradation of organic substrates, it is a common observation that oxygenation reactions are often plagued by undesired further transformation of the formed product, leading to low overall yields. As a consequence, selective blocking of subsequent metabolic steps (by leaving the cells intact) is the key to achieve high yields. Once this goal is achieved, biocatalytic oxygenation provides processes which are unparallelled to traditional oxidation methods. The latter has been achieved in the regio-selective oxidation of steroids based on a tremendous amount of research during the 1940s. Nowadays, virtually any center in a steroid can be selectively hydroxylated by choosing the appropriate microbial strain from an existing library (ref. 5).



progesterone
Rhizopus arrhizus
Aspergillus niger

nicotinic acid
Pseudomonas sp.

Fig. 10 Regioselective microbial hydroxylation performed on an industrial scale.

As a result, the regio-selective hydroxylation of steroids is performed on an industrial scale (Note ad). For example, hydroxylation of progesterone in the 11α-position by *Rhizopus arrhizus* (ref. 82) or *Aspergillus niger* made approximately half of the 37 steps of the conventional chemical synthesis redundant and made 11α-hydroxyprogesterone available for therapy at a reasonably cost (Fig. 10). Similarly, hydroxylation of nicotinic acid in position 6 is selectively achieved by using *Pseudomonas* sp. (ref. 83). For the average organic substrate, several useful candidates (usually fungi) (Note ae) are available, which can be tested for hydroxylation of unfunctionalized aliphatic moieties. Based on the results from structurally rigid substrates, models which allow the prediction of the outcome of an intended hydroxylation with respect to the stereochemistry of the product have been developed for the more extensively used microorganisms, such as *Beauveria bassiana* (ref. 84). However, despite this logic approach, microbial hydroxylation is still strongly empirical.

Microbial asymmetric epoxidation of alkenes (Note af) under the action of mono-oxygenases leads to the formation of chiral epoxides (ref. 85). The latter, however, exert a considerable toxicity to living cells, which are used for these biotransformations. This is mainly due to the fact that mono-oxygenases with epoxidizing activity are heme-dependent enzymes, which are sensitive to the alkylating properties of oxiranes. As a consequence, the concentration of the epoxide formed has to be kept to a low level, to ensure the viability of the biocatalyst, leading to a low overall productivity. Attempts to control the toxicity of epoxides by utilizing biphasic solvent systems consisting of water-immiscible organic solvents have been only partially successful due to damage of cell membranes caused by the bulk apolar phase. Since there is considerable success in the creation of synthetic chiral catalysts for the asymmetric epoxidation of alkenes such as the Sharpless or the Jacobson reagent, it remains to be seem whether biocatalytic methods will lead to a major breakthrough.

The asymmetric sulfoxidation of thioethers leads to the formation of chiral sulfoxides, which are highly valuable chiral intermediates (ref. 86). Like hydroxylation and epoxidation, these reactions are usually catalyzed by cofactor-requiring mono-oxygenases and, hence whole cell systems play a major role. Yields, however, are often better since the degradation of the substrate is more difficult as compared to the above

---

Note *ad*: Several other microbial processes showing an overall net-hydroxylation balance are proceeding through the β-oxidation pathway, *i.e.* dehydrogenation with subsequent addition of water, without a mono-oxygenase being involved. For instance, these cases apply to the production of both enantiomers of β-hydroxyisobutyric acid and L-carnitine.
Note *ae*: The most commonly used species belong to the genera: *Aspergillus*, *Beauveria*, *Cunninghamella*, *Rhizopus*, *Penicillium*. Bacteria (e.g. *Bacillus*, *Pseudomonas*, *Streptomyces*) are used to a lesser extent.
Note *af*: This activity is predominantly found in alkane- and alkene-utilizing bacteria, such as *Pseudomonas*, *Corynebacterium*, *Nocardia*, *Rhodococcus*, *Xanthobacter* and *Mycobacterium*.

mentioned oxidation processes. Furthermore, over-oxidation—which would lead to the formation of useless achiral sulfones—is generally not a major problem.

The oxidation of ketones to yield esters or lactones—the Baeyer-Villiger reaction—can be catalyzed by mono-oxygenase enzymes (refs. 87, 88). With whole-cell transformations, further degradation of the lactone such as hydrolytic opening may be efficiently controlled by addition of (highly toxic) lactonase-inhibitors. The strength of the biocatalytic Baeyer-Villiger oxidation lies in the (almost unique) possibility to perform these reactions in an asymmetric manner. However, some of the most widely used strains, such as *Acinetobacter calcoaceticus*, are class-II pathogens, which are not trivial to handle in an average laboratory on the large scale. This disadvantage has been overcome most recently by endowing an easy-to-use yeast strain with a Baeyer-Villiger capability using genetic engineering (ref. 89). It can be expected that this fascinating invention will boost the number of applications of microbial Baeyer-Villiger oxidation considerably.

The key step in the microbial degradation of aromatics by lower organisms, such as bacteria, is a *cis*-dihydroxylation leading to a chiral *cis*-glycol (Fig. 11). The latter reaction is catalyzed by di-oxygenases (Note ag). In wild-type organisms, the glycols are rapidly further oxidized by dihydrodiol dehydrogenases, involving re-aromatization of the diol intermediate with concomitant loss of chirality. Over the recent years, mutant strains with blocked dehydrogenase activity have been developed, which accumulate the glycols in the medium in high yield (Fig. 11) (ref. 90). Since the stereoselectivity is usually excellent, these *cis*-glycols have become very popular as highly functionalized starting materials for asymmetric synthesis (ref. 91). One particular trick, which provides access to both enantiomeric series of products, employs a single microbial strain but varies the R^2 group. This makes this strategy extremely flexible (Fig. 11). Based on the observation that the stereochemical outcome is mainly determined by the orientation of the groups R^1 and R^2 with respect to their relative size, an iodo-residue was temporarily introduced at position R^2 instead of the unsubstituted counterpart, leading to a formal inversion of the glycol moiety formed. Then, the directing iodo-group was reductively removed in a second step, leading to the formation of the enantiomeric product (ref. 92). Asymmetric dihydroxylation of aromatics is highly flexible as the group R^1 may be varied within a considerable range and also (hetero)cyclic derivatives may be employed. Several of these products are available on a kg-scale.

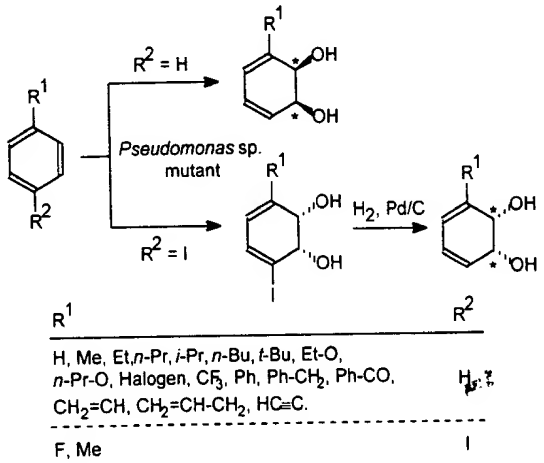


Fig. 11 Asymmetric dihydroxylation of aromatics with complementary selectivities.

Peroxidation

Oxidation reactions that consume hydrogen peroxide (Note ah) are catalyzed by peroxidases. At first glance, the absence of any cofactor such as NAD(P)H (which must be recycled) makes these enzymes superior to oxygenases. However, H_2O_2 exerts deleterious effects on proteins, in particular at higher concentrations. As a consequence, peroxidase reactions have to be performed with a low oxidant

Note *ag*: Named after their ability to incorporate both O-atoms from molecular oxygen into a substrate.
Note *ah*: In certain cases, hydrogen peroxide can be substituted by other related oxidants such as *tert*-butyl hydroperoxide, etc.

concentration to prevent enzyme deactivation thus limiting the productivity of the process. At present, this problem has not been adequately addressed from a preparative standpoint, but it might be solved by using H_2O_2-sensitive sensors coupled to an automatic addition of the oxidant. Until now the number of peroxidase-applications is remarkably low (ref. 93), but considerable developments such as epoxidation of alkenes, sulfoxidation of thioethers, etc., can be expected in this area (ref. 94).

ALDOL-TYPE REACTIONS

Asymmetric C-C bond formation based on catalytic aldol addition reactions remain a challenging subject in asymmetric synthesis. Although several successful non-biological approaches have been developed, they are not without drawbacks, such as (i) (often) stoichiometic in auxiliary reagent and (ii) the requirement for a metal or metal-like enolate complex. Due to the instability of these latter reagents, the reactions have to be performed in organic solvents at low temperature. Furthermore, any additional polar functional groups—*e.g.* in carbohydrates—present in the molecule have to be protected for solubility reasons and to avoid undesired cross-reactions. In contrast, since Nature has developed enzymes which can handle carbanionic intermediates in water, biocatalytic aldol reactions can be performed in aqueous solution at neutral pH without need for extensive protection methodology (ref. 95). In general, aldolases are very faithful for their donor (the carbanion equivalent) but possess relaxed specificities for the acceptor (the carbonyl component).

The first group of enzymes having great potential in this respect are aldolases. These widely used enzymes catalyze the addition of a C_3-carbon unit (*i.e.* dihydroxyacetone phosphate, DHAP) on an aldehyde with the simultaneous creation of two new stereogenic centers with (usually) excellent enantio- and diastereo-selectivity. The full synthetic potential of DHAP-dependent aldolases depends on the availability of the complete set of enzymes providing access to products with complementary stereochemistry. As a result of intense research over the past years, this quartet of enzymes is now available (Fig. 12) (ref. 95).

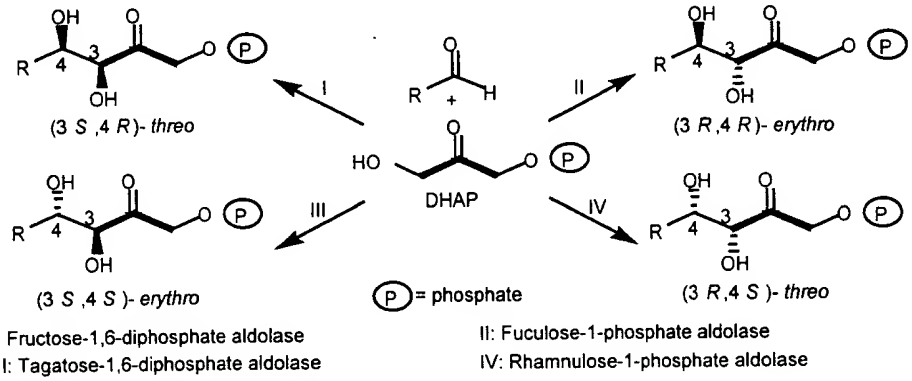


Fig. 12 Complementary stereochemistry of DHAP-dependent aldolases.

One general problem associated with the aldolases mentioned above originates from their requirement for a phosphorylated donor, *i.e.* DHAP. Although chemical or biological synthesis is possible, DHAP is not particularly stable (Note ai) and its synthesis is not trivial. Since in the majority of cases the (phosphorylated) products have to be dephosphorylated in a subsequent enzymatic step to obtain the desired target molecule, the availability of enzymes which depend on non-phosphorylated donors would be of a great advantage. In view of this goal, current research on aldol-type reactions has focused on two directions, *i.e.* (i) aldolases accepting pyruvate (ref. 96), acetaldehyde (ref. 97) or glycine (ref. 98) and (ii) transketolase-enzymes dependent on hydroxypyruvate (refs. 99, 100). Significant advances can be expected in the near future.

Note *ai*: Half life time in aqueous solution at pH 7 about 20 h.

ADDITION AND ELIMINATION

The asymmetric addition of small molecules such as water or ammonia to C=C bonds or hydrogen cyanide onto C=O bonds is catalyzed by lyases. During such a reaction one (or, depending on the substitution pattern of the substrate, two) stereogenic centers are created. With the exception of cyanohydrin formation, the synthetic potential of these reactions is restricted due to the fact that lyases generally exhibit narrow specificities and thus only minor structural variations to their natural substrate(s) are allowed.

Hydroxynitrile lyases catalyze the asymmetric cyanohydrin formation from hydrogen cyanide and an aldehyde or—less common—a ketone (refs. 101, 102). The latter are versatile starting materials for the synthesis of several types of compounds such as α-hydroxyacids or -esters, acyloins or 1,2-diols, ethanolamines as well as amino-nitriles and aziridines (ref. 101). Furthermore, some chiral cyanohydrins constitute the alcohol moieties of commercial pyrethroid insecticides such as Deltamethrin and Fluvalinate. As the cyanohydrin formation constitutes an asymmetrisation reaction providing one product enantiomer in 100% theoretical yield, the availability of enzymes having opposite stereochemical preference is of great importance. Whereas (*R*)-hydroxynitrile lyases (Note aj) have been thoroughly investigated, research on the complementary (*S*)-specific enzymes (Note ak) started more recently (ref. 103). The top-candidates for synthetic biotransformations are the (*R*)-specific almond enzyme and the (*S*)-selective counterpart from the Brasilian gum tree. Whereas the former can be cheaply obtained from natural sources, the latter has been made available through genetic engineering. Biocatalytic cyanohydrin formation has been well researched and can be considered as a reliable method. Some contemporary limitations with respect to the availability of (*S*)-enzymes are expected to be overcome soon.

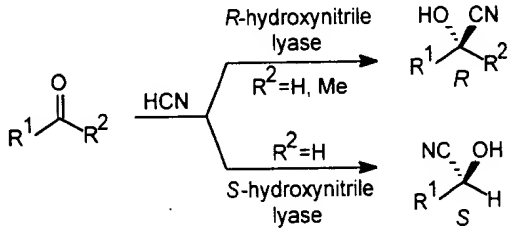


Fig. 13 Biocatalytic cyanohydrin formation with complementary stereochemistry.

NOVEL TECHNIQUES

At present, the research goals within the area of biotransformations are undergoing a rapid change. The majority of biotransformations involving chiral molecules are performed with racemic substrates rather than prochiral or *meso*-forms (Note al)—*i.e.* they constitute kinetic resolutions as opposed to asymmetrizations. The former, however, are impeded by several drawbacks such as (i) limited yield (50% of each enantiomer), (ii) reduced enantiomeric purity of substrate and product for kinetic reasons, (iii) close reaction monitoring required, because the process has to be stopped at a certain point of conversion at (or near) 50% and (iv) separation of the product formed from unreacted substrate. All of these impediments are largely circumvented by using processes which lead to the formation of a single enantiomeric product. As a consequence, the theoretical yield is now 100% with optical purities being higher, the separation step is obsolete and monitoring can be simplified because the reaction can be run to completion.

Such processes are, for instance, (i) dynamic resolutions and (ii) stereo-inversions (Fig. 14) (ref. 104). Any kinetic resolution of a racemic starting material (R, S) following a classic profile can be converted into a dynamic process by adding one additional reaction—an *in-situ*-racemization of the substrate. While in classic resolutions the fast reacting enantiomer (*e.g.* R) is transformed into P leaving its enantiomer S behind, in a dynamic process S is converted to P *via* racemization. As a consequence, all of the starting material (R, S) is eventually transformed into P as the sole product. The dynamic resolution processes developed so far made use of racemization through chemical catalysis, but it can be expected that two-

Note *aj*: (*R*)-Hydroxynitrile lyases are available predominantly from plants of the *Rosacea* family, *e.g.* almond, plum, cherry, apricot, etc.
Note *ak*: (*S*)-Hydroxynitrile lyases were obtained from *Sorghum bicolor* (millett), *Hevea brasiliensis* (gum tree), *Ximenia americana* (sandalwood), *Sambucus nigra* (elder) and *Manihot esculenta* (cassava).
Note *al*: The ratio of kinetic resolution versus asymmetrization is about 4:1 (Database Faber, 05/1997).

enzyme processes involving racemases are possible. The latter show great potential, bearing in mind the greater compatibility of biocatalysts as opposed to the combination of chemo- and biocatalysis.

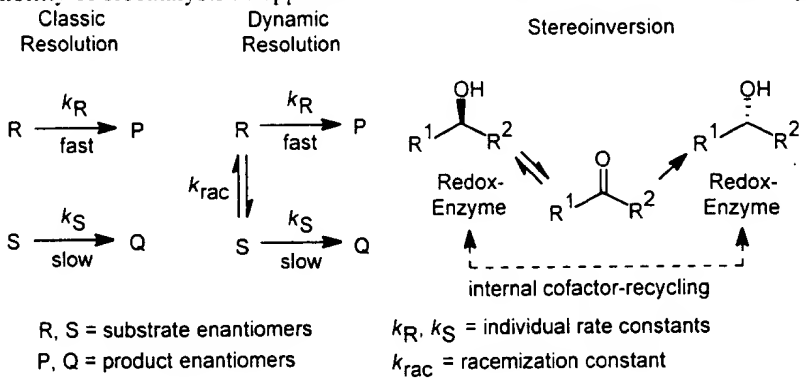


Fig. 14 Dynamic resolution and stereo-inversion processes.

A particular reaction sequence leading to the formation of a single enantiomeric product makes use of an enantioselective stereoinversion (ref. 104). Suppose that one enantiomer out of a racemic mixture is selectively inverted into its mirror-image counterpart, the final product is 100% of one enantiomer. This type of process has been exemplified with the stereoinversion of racemic secondary alcohols through a two-step oxidation-reduction sequence using microbial whole cells (ref. 105). Although the actual nature of the enzymes and cofactors involved is not known at present, the chemical and optical yields achieved are impressive. It can be anticipated, that once the catalytic and thermodynamic details about this sequence are known, more synthetic applications will appear.

The methodology concerning the application of enzymes in non-aqueous solvents with respect to enzyme *activity* is sufficiently understood as to be highly useful for organic chemists. Thus, the synthesis of esters, lactones, amides, peptides and peracids by using enzymes is standard methodology. On the other hand, an understanding of the influence of the nature of the solvent on an enzyme's *selectivity* is still in its infancy and we are far away from being able to provide rules of general applicability (ref. 28). The same is true with regard to the understanding of the factors involved in substrate-binding where assistance will be given by means of molecular modelling (ref. 106).

SUMMARY AND OUTLOOK

The strengths and weaknesses of the most widely used enzyme systems are summarized in Table 1. Possible solutions to existing problems are addressed in the respective column. As the field of biocatalysis undergoes still fast development, it can be expected, that some of the limitations will be overcome in the near future by using novel (genetically engineered) enzymes and through the development of novel techniques.

Despite tremendous efforts in the field of genetic engineering (such as site-directed mutagenesis) aimed at the development of enzymes possessing altered specificities it is clear that at this stage, screening for novel microorganisms and enzymes is much more likely to lead to a solution of a particular problem than painstaking modification of the enzyme. After all, enzymes have been optimizing their skills for more than 3×10^9 years in order to develop a lot of sophisticated chemistry, whereas organic chemists have a track-record of less than a century. Fortunately there is some evidence that the number of distinctly different enzyme mechanisms is finite, since we already know examples of enzyme molecules that are unrelated by evolution but possess almost identically arranged functional groups. In these cases Nature has obviously faced the same biochemical problem and has found the same optimum solution. Of more immediate use is the cloning and over-expression of enzymes in sturdy and easy-to-cultivate host organisms (for those cases where the enzymes are found only in trace amounts in sensitive microorganisms) thus providing sufficient quantities of biocatalysts for biotransformations on a synthetic scale.

Table 1. Pros and cons of biotransformations according to enzyme types

Enzyme Type	Reaction Catalyzed	Strength	Weakness	Solution
lipase	ester hydrolysis, -formation, -aminolysis	many stable enzymes organic solvents	low predictability for stereoselectivity	state of the art, case closed?
esterase	ester hydrolysis, -formation	pig liver esterase proteases	few esterases organic solvents	novel esterases?
protease	ester & amide hydrolysis, ester aminolysis, peptide synthesis	many stable proteases	no D-proteases	genetically engineered D-proteases?
nitrilase, nitrile hydratase	nitrile hydrolysis	chemo- and regio-selectivity	enantioselectivity, no commercial enzyme(s)	new commercial nitrilases?
epoxide hydrolase phosphatase/kinase haloalkane dehalogenase	hydrolysis of epoxides, phosphate esters haloalkanes	chemocatalysis fails	few enzymes	new (commercial) enzymes?
glycosidase	oligosaccharide formation	anomeric selectivity	regio- and diastereo-selectivity limited yields	state of the art, case closed?
dehydrogenase	reduction of aldehydes + ketones	Prelog-selectivity NADH-enzymes whole cells	anti-Prelog-selectivity, NADPH-enzymes	NADH-dependent anti-Prelog enzymes, PQQ-enzymes?
enoate reductase	reduction of enones, α,β-unsaturated esters	whole-cell systems	isolated enzymes	state of the art, case closed?
mono-oxygenase	hydroxylation, Baeyer-Villiger, epoxidation	chemocatalysis fails	multi-component sensitive NADPH-enzymes	non-heme, NADH-enzymes?
di-oxygenase	dihydroxylation of aromatics	whole-cell systems	isolated enzymes	state of the art, case closed?
peroxidase	peroxidation, epoxidation	no cofactor	H_2O_2-sensitive	new enzymes?
aldolase	aldol reaction in H_2O	stereo-complementary DHAP-enzymes	dihydroxyacetone phosphate	non-phosphorylated donors?
transketolase	transketolase reaction	non-phosphorylated donors	few enzymes	new enzymes?
hydroxynitrile lyase	cyanohydrin formation	*R*- & *S*-enzymes	few enzymes	commercial enzymes?
fumarase, aspartase	addition of H_2O, NH_3	chemocatalysis fails	narrow substrate tolerance	new enzymes, case closed?
glycosyl transferase	oligosaccharide synthesis	high selectivity	phosphorylated donors	state of the art, case closed?

In summary, biocatalysts represent a new class of chiral catalysts useful for a broad range of selective organic transformations. Synthetic chemists capable of using this potential will have a clear advantage over those limited to non-biological methods in their ability to tackle the new generation of synthetic problems appearing at the interface between chemistry and biology.

ACKNOWLEDGEMENT

The author wishes to express his cordial thanks to A. Kiener (Visp, Switzerland) and D. Johnson (Graz) for helpful hints and discussions.

REFERENCES

1. S. G. Neidleman. In *Biocatalysis*. (D. Abramowicz, ed.), pp. 1–24, Van Nostrand Reinhold, New York (1990).
2. S. M. Roberts, K. Wiggins and G. Casy. (eds.) *Preparative Biotransformations: Whole Cells and Isolated Enzymes*, Wiley, Chichester (1993).
3. S. M. Roberts, N. J. Turner, A. J. Willetts and M. K. Turner. *Introduction to Biocatalysis Using Enzymes and Micro-organisms*, Cambridge University Press, Cambridge (1995).

4. J. B. Jones, C. J. Sih and D. Perlman. (eds) *Applications of Biochemical Systems in Organic Chemistry*, 2 parts, Wiley, New York (1976).
5. H. G. Davies, R. H. Green, D. R. Kelly and S. M. Roberts. *Biotransformations in Preparative Organic Chemistry*, Academic Press, London (1989).
6. J. S. Dordick. (ed) *Biocatalysts for Industry*, Plenum Press, New York (1991).
7. J. Halgas. *Biocatalysts in Organic Synthesis*, Studies in Organic Chemistry, vol. **46**, Elsevier, Amsterdam (1992).
8. A. N. Collins, G. N. Sheldrake and J. Crosby. (eds) *Chirality in Industry*, Wiley, Chichester (1992).
9. L. Poppe and L. Novak. *Selective Biocatalysis*, Verlag Chemie, Weinheim (1992).
10. J. M. S. Cabral, D. Best, L. Boross and J. Tramper. (eds) *Applied Biocatalysis*, Harwood, Chur (1994).
11. K. Kieslich. *Microbial Transformations of Non-Steroid Cyclic Compounds*, Thieme, Stuttgart (1976).
12. K. Drauz and H. Waldmann. (eds) *Enzyme Catalysis in Organic Synthesis*, 2 vols., Verlag Chemie, Weinheim, (1995).
13. C. Crosby. *In Chirality in Industry* (A. N. Collins, G. N. Sheldrake and J. Crosby, eds.), pp. 1–66. Wiley, Chichester (1992).
14. K. Faber. *Biotransformations in Organic Chemistry—A Textbook*, 3rd edn., Springer, Heidelberg (1997).
15. P. Feyerabend. *Against Method*, Verso, London (1988).
16. K. Faber and M. C. R. Franssen. *Trends Biotechnol.* **11**, 461–470 (1993).
17. M. K. Turner. *Trends Biotechnol.* **13**, 253–258 (1995).
18. M. Hanson. *Oils & Fats Int.* (5) 29–32 (1990).
19. W. Boland, C. Frößl and M. Lorenz. *Synthesis*, 1049–1072 (1991).
20. R. J. Kazlauskas, A. Weissfloch, A. T. Rappaport and L. A. Cuccia. *J. Org. Chem.* **56**, 2656–2665 (1991).
21. A. N. E. Weissfloch and R. Kazlauskas. *J. Org. Chem.* **60**, 6959–6969 (1995).
22. E. Santaniello, P. Ferraboschi and P. Grisenti. *Enzyme Microb. Technol.* **15**, 367–382 (1993).
23. K. Faber and S. Riva. *Synthesis* 985–910 (1992).
24. M. J. Garcia, F. Rebolledo and V. Gotor. *Tetrahedron* **50**, 6935–6940 (1994).
25. C. Astorga, F. Rebolledo and V. Gotor. *Synthesis* 287–289 (1993).
26. F. Björkling, H. Frykman, S. E. Godtfredsen and O. Kirk. *Tetrahedron* **48**, 4587–4592 (1992).
27. C.-S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih. *J. Am. Chem. Soc.* **104**, 7294–7299 (1982).
28. G. Carrea, G. Ottolina and S. Riva. *Trends Biotechnol.* **13**, 63–70 (1995).
29. C. Laane, S. Boeren, K. Vos and C. Veeger. *Biotechnol. Bioeng.* **30**, 81–87 (1987).
30. Y. Hirose, K. Kariya, I. Sasaki, Y. Kurono, H. Ebiike and K. Achiwa. *Tetrahedron Lett.* **33**, 7157–7160 (1992).
31. K. Faber, G. Ottolina and S. Riva. *Biocatalysis* **8**, 91–132 (1993).
32. J. B. Jones. *Pure Appl. Chem.* **62**, 1445–1448 (1990).
33. M. Ohno and M. Otsuka. *Org. React.* **37**, 1–55 (1989).
34. L. R. Randrianasolo-Rokotozafy, R. Azerad, F. Dumas, D. Potin and J. d'Angelo. *Tetrahedron: Asymmetry* **4**, 761–766 (1993).
35. L. Heiss and H.-J. Gais. *Tetrahedron Lett.* **36**, 3833–3836 (1995).
36. C. R. Johnson, J. P. Adams, S. J. Bis, R. L. De Jong, A. Golebiowski, J. R. Medich, T. D. Penning, C. H. Senanayake, D. H. Steensma and M. C. Van Zandt. *Indian J. Chem., Sect. B*, **32B**, 140–144 (1993).
37. R. Chenevert and R. Martín. *Tetrahedron: Asymmetry* **3**, 199–200 (1992).
38. H. Kotani, Y. Kuze, S. Uchida, T. Miyabe, T. Iimori, K. Okano, S. Kobayashi and M. Ohno. *Agric. Biol. Chem.* **47**, 1363–1365 (1983).
39. W. J. Quax and C. P. Broekhuizen. *Appl. Microbiol. Biotechnol.* **41**, 425–431 (1994).
40. J. W. H. Smeets and A. P. G. Kieboom. *Recl. Trav. Chim. Pays-Bas* **111**, 490–495 (1992).
41. O. Francetic, V. Deretic, N. Marjanovic and V. Glisin. *Biotech-Forum* **5**, 90–94 (1988).
42. J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker and I. Silman. *Science* **253**, 872–879 (1991).
43. R. M. Williams. *Synthesis of Optically Active α-Amino Acids*. Pergamon Press, Oxford (1989).

44. J. Kamphuis, E. M. Meijer, W. H. J. Boesten, Q. B. Broxterman, B. Kaptein, H. F. M. Hermes and H. E. Schoemaker. In *Production of Natural and Synthetic L- and D-Amino Acids by Aminopeptidases and Amino Amidases*. (J. D.Rozzell and F. Wagner, eds.), pp. 177–206, Hanser, Munich (1992).
45. A. S. Bommarius, M. Schwarm and K. Drauz. *Chimica Oggi*, 61–64 (1996).
46. W. Kullmann. *Enzymatic Peptide Synthesis*. CRC Press, Boca Raton (1987).
47. V. Schellenberger and H.-D. Jakubke. *Angew. Chem., Int. Ed. Engl.* **103**, 1437–1449 (1991).
48. S. Gerisch, H.-D. Jakubke and H.-J. Kreuzfeld. *Tetrahedron: Asymmetry* **6**, 3039–3045 (1995).
49. J. B. West and C.-H. Wong. *Tetrahedron Lett.* **28**, 1629–1632 (1987).
50. H. K. Chenault, J. Dahmer and G. M. Whitesides. *J. Am. Chem. Soc.* **111**, 6354–6364 (1989).
51. T. Nagasawa and H. Yamada. *Pure Appl. Chem.* **62**, 1441–1444 (1990).
52. O. Meth-Cohn and M.-X. Wang. *Tetrahedron Lett.* **36**, 9561–9564 (1995).
53. H. Yamada. *Chimia* **47**, 69–74 (1993).
54. A. Kiener. *CHEMTECH* **25**, 31–35 (1995).
55. M. Kobayashi, T. Nagasawa and H. Yamada. *Appl. Microbiol. Biotechnol.* **29**, 231–233 (1988).
56. M. Yokoyama, T. Sugai and H. Ohta. *Tetrahedron: Asymmetry* **4**, 1081–1084 (1993).
57. H. C. Kolb, M. S. VanNieuwenhze and K. B. Sharpless. *Chem. Rev.* **94**, 2483–2547 (1994).
58. F. Oesch. *Xenobiotica* **3**, 305–340 (1972).
59. K. Faber, M. Mischitz and W. Kroutil. *Acta Chem. Scand.* **50**, 249–258 (1996).
60. S. Pedragosa-Moreau, A. Archelas and R. Furstoss. *J. Org. Chem.* **58**, 5533–5536 (1993).
61. R. V. A. Orru, W. Kroutil and K. Faber. *Tetrahedron Lett.* **38**, 1753–1754 (1997).
62. S. Pedragosa-Moreau, A. Archelas and R. Furstoss. *Tetrahedron* **52**, 4593–4606 (1996).
63. I. Osprian, W. Kroutil, M. Mischitz and K. Faber. *Tetrahedron: Asymmetry* **8**, 65–71 (1997).
64. G. Bellucci, C. Chiappe and A. Cordoni. *Tetrahedron: Asymmetry* **7**, 197–202 (1996).
65. M. Schultz, H. Waldmann, H. Kunz and W. Vogt. *Liebigs Ann. Chem.* 1019–1024 (1990).
66. H. K. Chenault, E. S. Simon and G. M. Whitesides. *Biotechnol. Genet. Eng. Rev.* **6**, 221–270 (1988).
67. C.-H. Wong, S. L. Haynie and G. M. Whitesides. *J. Am. Chem. Soc.* **105**, 115–117 (1983).
68. M. Onda, K. Motosugi and H. Nakajima. *Agric. Biol. Chem.* **54**, 3031–3033 (1990).
69. S. C. Taylor. In *Opportunities in Biotransformations*. (L. G. Copping, R. E. Martin, J. A. Pickett, C. Bucke and A. W. Bunch. eds.), pp. 170–176, Elsevier, London (1990).
70. T. Nakamura, F. Yu, W. Mizunashi and I. Watanabe. *Agric. Biol. Chem.* **55**, 1931–1933 (1991).
71. K. G. I. Nilsson. *Trends Biotechnol.* **6**, 256–264 (1988).
72. K. G. I. Nilsson. *Carbohydr. Res.* **204**, 79–83 (1990).
73. E. N. Vulfson, R. Patel, J. E. Beecher, A. T. Andrews and B. A. Law. *Enzyme Microb. Technol.* **12**, 950–954 (1990).
74. D. H. G. Crout, D. A. MacManus and P. Critchley. *J. Chem. Soc., Perkin Trans. 1*, 1865–1868 (1990).
75. W. Hummel and M.-R. Kula. *Eur. J. Biochem.* **184**, 1–13 (1989).
76. S. Servi. *Synthesis* 1–25 (1990).
77. V. Prelog. *Pure Appl. Chem.* **9**, 119–130 (1964).
78. W. Hummel, H. Schütte, E. Schmidt, C. Wandrey and M.-R. Kula. *Appl. Microbiol. Biotechnol.* **26**, 409–416 (1987).
79. K. Seelbach, B. Riebel, W. Hummel, M.-R. Kula, V. I. Tishkov, A. M. Egorov, C. Wandrey and U. Kragl. *Tetrahedron Lett.* **37**, 1377–1380 (1996).
80. H. Simon. *Indian J. Chem., Sect. B*, **32B**, 170–175 (1993).
81. H. L. Holland. *Organic Synthesis with Oxidative Enzymes*. Verlag Chemie, Weinheim (1992).
82. D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh. *J. Am. Chem. Soc.* **74**, 5933–5936 (1952).
83. H. G. Kulla. *Chimia* **45**, 81–85 (1991).
84. R. Furstoss, A. Archelas, J. D. Fourneron and B. Vigne. In *Enzymes as Catalysts in Organic Synthesis* (M. P. Schneider, ed.), NATO ASI Series C, vol. **178**, pp. 361–70, Reidel, Dordrecht (1986).
85. K. Furuhashi. *Chem. Econ. Eng. Rev.* **18**, (7–8), 21–26 (1986).

86. R. S. Phillips and S. W. May. *Enzyme Microb. Technol.* **3**, 9–18 (1981).
87. S. M. Roberts and A. J. Willetts. *Chirality* **5**, 334–337 (1993).
88. G. Ottolina, G. Carrea, S. Colonna and A. Rückemann. *Tetrahedron: Asymmetry* **7**, 1123–1136 (1996).
89. J. D. Stewart, K. W. Reed and M. M. Kayser. *J. Chem. Soc., Perkin Trans. 1*, 755–757 (1996).
90. D. T. Gibson, J. R. Koch and R. E. Kallio. *Biochemistry* **7**, 2653–2662 (1968).
91. T. Hudlicky, K. A. Abboud, D. A. Entwistle, R. Fan, R. Maurya, A. J. Thorpe, J. Bolonick and B. Myers. *Synthesis* 897–911 (1996).
92. C. C. R. Allen, D. R. Boyd, H. Dalton, N. D. Sharma, I. Brannigan, N. A. Kerley, G. N. Sheldrake and S. S. Taylor. *J. Chem. Soc., Chem. Commun.* 117–118 (1995).
93. V. K. Datcheva, K. Kiss, L. Solomon and K. S. Kyler. *J. Am. Chem. Soc.* **113**, 270–274 (1991).
94. M. C. R. Franssen. *Biocatalysis* **10**, 87–111 (1994).
95. W.-D. Fessner and C. Walter. *Topics Curr. Chem.* **184**, 97–194 (1996).
96. C.-H. Lin, T. Sugai, R. L. Halcomb, Y. Ichikawa and C.-H. Wong. *J. Am. Chem. Soc.* **114**, 10138–10145 (1992).
97. C. F. Barbas III, Y.-F. Wang and C.-H. Wong. *J. Am. Chem. Soc.* **112**, 2013–2014 (1990).
98. V. P. Vassilev, T. Uchiyama, T. Kajimoto and C.-H. Wong. *Tetrahedron Lett.* **36**, 4081–4084 (1995).
99. Y. Kobori, D. C. Myles and G. M. Whitesides. *J. Org. Chem.* **57**, 5899–5907 (1992).
100. K. G. Morris, M. E. B. Smith, N. J. Turner, M. D. Lilly, R. K. Mitra and J. M. Woodley. *Tetrahedron: Asymmetry* **7**, 2185–2188 (1996).
101. F. Effenberger. *Angew. Chem., Int. Ed. Engl.* **106**, 1555–1565 (1994).
102. G. J. M. van Scharrenburg, J. B. Sloothaak, C. G. Kruse, E. Smitskamp-Wilms and J. Brussee. *Indian J. Chem., Sect. B*, **32B**, 16–19 (1993).
103. N. Klempier, U. Pichler and H. Griengl. *Tetrahedron: Asymmetry* **6**, 845–848 (1995).
104. K. Faber and H. Stecher. *Synthesis* 1–16 (1997).
105. J. Hasegawa, M. Ogura, S. Tsuda, S. Maemoto, H. Kutsuki and T. Ohashi. *Agric. Biol. Chem.* **54**, 1819–1827 (1990).
106. R. J. Kazlauskas. *Trends Biotechnol.* **12**, 464–472 (1994).